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Proposed Registration Decision PRD2015-11

Pasteuria nishizawae Pn1

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Overview

Proposed Registration Decision for *Pasteuria nishizawae* Pn1

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Pasteuria nishizawae* Technical and Clariva pn, containing the technical grade active ingredient *Pasteuria nishizawae* Pn1, to suppress soybean cyst nematode in soybean.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of *Pasteuria nishizawae* Technical and Clariva pn.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment. These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides and Pest Management portion of Health Canada's website at healthcanada.gc.ca/pmra.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act*.

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (*a*) efficacy; (*b*) effect on host organisms in connection with which it is intended to be used; and (*c*) health, safety and environmental benefits and social and economic impact."

Before making a final registration decision on *Pasteuria nishizawae* Pn1, the PMRA will consider all comments received from the public in response to this consultation document.[3] The PMRA will then publish a Registration Decision[4] on *Pasteuria nishizawae* Pn1, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What Is Pasteuria nishizawae Pn1?

Pasteuria nishizawae Pn1 is a mycelial and endospore-forming bacterium, naturally found in North American soils, which parasitizes the adult females of cyst-forming nematodes, such as the soybean cyst nematode (SCN - *Heterodera glycines*).

Health Considerations

Can Approved Uses of *Pasteuria nishizawae* Pn1 Affect Human Health?

***Pasteuria nishizawae* Pn1 is unlikely to affect your health when Clariva pn is used according to the label directions.**

People could be exposed to *P. nishizawae* Pn1 when handling and applying Clariva pn. When assessing health risks, several key factors are considered:

- the microorganism's biological properties (for example, infectivity cycle);
- reports of any adverse incidents;
- its potential to cause disease or toxicity as determined in toxicological studies; and
- the level to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns. When *Pasteuria nishizawae* Technical and Clariva pn were tested on laboratory animals, there were no signs that it caused any significant toxicity or disease.

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3 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

4 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

Residues in Water and Food

Dietary risks from food and water are not of concern.

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally specified as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada specifies science-based MRLs to ensure that the food Canadians eat is safe.

Residues of *P. nishizawae* Pn1 are not expected on crops at the time of harvest following seed treatment. When *Pasteuria nishizawae* Technical and Clariva pn, which contain spores of *P. nishizawae* Pn1 as the active ingredient, were administered orally to rats, no signs of toxicity or disease were observed. Dietary exposure is expected to be negligible and the likelihood of residues contaminating drinking water supplies is also considered to be negligible. Consequently, dietary risks are not of concern. Therefore, the PMRA has determined that the specification of an MRL under the *Pest Control Products Act* is not required for *P. nishizawae* Pn1.

Risks in Residential and Other Non-Occupational Environments

Estimated risk for non-occupational exposure is not of concern.

Clariva pn is proposed for use as a seed treatment on soybeans. Consequently, it is unlikely that adults, youths and toddlers will be exposed to *P. nishizawae* Pn1. Even in the event of exposure, risk to the general population is not of concern since there were no signs of disease or toxicity noted in mammalian toxicological studies conducted with the technical and end-use product.

Occupational Risks From Handling Clariva pn

Occupational risks are not of concern when Clariva pn is used according to label directions, which include protective measures

Workers handling Clariva pn can come into direct contact with *P. nishizawae* Pn1 on the skin, eyes and lungs. For this reason, the product label will specify that workers exposed to Clariva pn must wear waterproof gloves, long-sleeved shirts, long pants, shoes plus socks, and a NIOSH approved mist filtering mask or respirator with any N-95, P-95, or R-95 filter. Eye goggles are not required, as the eye irritation studies submitted indicated no eye irritation potential.

For the bystander, exposure is expected to be much less than that of handlers and mixer/loaders and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When *Pasteuria nishizawae* Pn1 Is Introduced Into the Environment?

Environmental risks are not of concern

Pasteuria nishizawae Pn1 is a spore producing bacterium that is ubiquitous in terrestrial soils, as are other members of the genus. The spores are expected to be stable in a wide range of environmental conditions and are host specific; they will only parasitize SCN.

Clariva pn is not intended for aquatic uses and exposure to aquatic environments is expected to be minimal.

Although non-target testing was not conducted, acceptable scientific rationales were used to determine that no significant adverse effects to non-target organisms are expected.

Value Considerations

What Is the Value of Clariva pn?

Clariva pn, containing *Pasteuria nishizawae* Pn1, is a soybean seed treatment product used for the suppression of soybean cyst nematode.

This microbial product provides an additional tool to be used in conjunction with existing integrated pest management strategies such as using soybean cyst nematode (SCN) resistant varieties and rotation with other crops such as corn. Clariva pn is compatible with certain other chemical seed treatments and with *Rhizobium* spp. based inoculants.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the label of Clariva pn to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

In individuals exposed repeatedly to potentially large quantities of Clariva pn, respiratory and dermal sensitivity may possibly develop. All microorganisms, including *P. nishizawae* Pn1, contain substances that are potential sensitizers. Therefore, anyone handling or applying Clariva pn must wear appropriate waterproof gloves, a long-sleeved shirt, long pants, shoes plus socks, and a NIOSH approved mist filtering mask or respirator with any N-95, P-95 or R-95

filter. Also, the signal words, "**POTENTIAL SENSITIZER**" on the principal display panel and precautionary statements, "May cause sensitization" are required on the secondary display panel of the label for Clariva pn.

Environment

The end-use product label will include environmental precaution statements that prevent the contamination of aquatic systems from the use of Clariva pn.

Next Steps

Before making a final registration decision on *Pasteuria nishizawae* Pn1, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Pasteuria nishizawae* Pn1 (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Pasteuria nishizawae Pn1

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active ingredient	Live spores of *Pasteuria nishizawae* Pn1
Function	Nematicide to suppress soybean cyst nematode (*Heterodera glycines*) in soybeans
Binomial name	*Pasteuria nishizawae* Pn1
Taxonomic designation[1]	
Kingdom	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Pasteuriaceae
Genus	*Pasteuria*
Species	*nishizawae*
Isolate	Pn1
Patent Status information	None
Nominal purity of active	Technical Grade Active Ingredient (TGAI): 100% w/w spores of *Pasteuria nishizawae* strain Pn1, > 1.0×10^{10} spores/gram End-Use Product (EP): 15% w/w *Pasteuria nishizawae* Technical, > 1.58×10^{10} spores/mL (minimum)
Identity of relevant impurities of toxicological, environmental and/or significance.	The TGAI does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards. In addition, there are no known mammalian toxins or other known toxic metabolites present in the TGAI or EP.

[1] http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi

3.2 Physical and Chemical Properties of the Active Ingredients and End-Use Product

Technical Product — *Pasteuria nishizawae* Technical

Property	Result
Physical state	Solid
Density/Relative density/Bulk density	0.25 g/mL
Viscosity	Not applicable
Corrosion characteristics	As a microbial fermentation product, the PN-Nf is not expected to be an oxidizer and will not be capable of reacting exothermically with combustible materials

End-Use Product — Clariva pn

Property	Result
Colour	Tan
Physical state	Liquid
Density/Relative density/Bulk density	1.08 g/mL
Viscosity	210.2 cSt at 20°C
Corrosion characteristics	Clariva pn showed no signs of corrosion or deterioration after 12 months of storage

3.3 Directions for Use

Clariva pn is a nematicide seed treatment. It is diluted in water and applied directly to dry soybean seeds, for the suppression of soybean cyst nematode (*SCN* = *Heterodera glycines*). Clariva pn has a variable guarantee, with a minimum of 1.5×10^{10} spores per mL. Clariva pn is applied at a rate of 10 million spores per seed. The amount of product (mL) applied per 100 kg seed is dependent upon the guarantee of each container and the seed size.

3.4 Mode of Action

After the nematode hatches from the egg, contact with *P. nishizawae* Pn1 nematode endospores can occur. These endospores attach to the body of the nematode and begin to germinate inside of the nematode. Mycelia develop and the sporulation process begins. As the nematode matures, the endospores mature and fill the nematode body. Females that are parasitized typically produce little to no eggs, ultimately reducing the SCN population. A portion of the nematodes colonized by *P. nishizawae* Pn1 may also be weakened and will not have the ability to enter the soybean root system. Upon nematode death and rupture or disintegration of the diseased cyst, more endospores are released which can come into contact with other nematodes, further reducing the loading of SCN on soybean roots.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganisms

A molecular biological analysis using 16S rRNA sequences can be performed to demonstrate it is a *Paraburkholderia spp. Paraburkholderia rhizoxinica* Rh1 can be identified to the species level most reliably by using two bioassays for host specificity. In one bioassay, spores of *P. rhizoxinica* Rh1 are assayed against cultures of the host nematode *M. ghorei* and observed for spore attachment and spore viability in the host. In a second bioassay, spores of *P. rhizoxinica* Rh1 are assayed against cultures of the host nematode *M. ghorei* as well as other nematodes susceptible to infection by other *Paraburkholderia spp.* and then observed for spore attachment. These methods reliably identify the microbial pest control agent (MPCA) since spores of *P. rhizoxinica* Rh1will only germinate in nematodes in the genus *Mesorhabditis*.

2.2 Methods for Establishment and Purity of Seed Stock

The production strain is maintained as master seed stock cultures and working stock cultures that are stored at -80°C. Practices for ensuring the purity and the integrity of the master seed stock and working stock cultures were adequately described in the method of manufacture and quality assurance program.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material and for the Production of Pre-analyzed Products

The guarantee of the technical is expressed as the number of spores per gram. Representative data on a five batches of *Paraburkholderia rhizoxinica* Technical were submitted.

The guarantee of the end use product is expressed as the number of spores per mL. This guarantee is calculated from % w/w content of *Paraburkholderia rhizoxinica* Technical in Clariva pn.

Representative data included spore counts.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

As noted in Section 2.1, the MPCA can be identified to the species level using host specificity bioassays. No methods are required to quantify viable or non-viable residues of *P. rhizoxinica* Rh1. *Paraburkholderia rhizoxinica* is a ubiquitous microorganism in nature and has been isolated from a wide variety of soils. The use of Clariva pn is not expected to significantly increase the natural environmental background levels of this microorganism. Furthermore, when *P. rhizoxinica* Rh1 was administered orally to rats, no signs of toxicity or disease were observed.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures that will be used to limit contaminating microorganisms during manufacture of *Paraburkholderia rhizoxinica* Technical and Clariva pn are acceptable.

During manufacturing, several approaches are used to limit microbial contamination in the technical grade active ingredient and end-use product. These approaches will include monitoring for signs of contamination and plating on selective agar media.

The absence of human pathogens and below-threshold levels of contaminating microorganisms were shown in the microbial screening of production batches using microbe-specific screening methods for detecting and enumerating microbial contaminants of concern. Release standards for microbial contaminants comply with those permitted by the PMRA and are adequate to ensure that the end-use product does not contain unacceptable levels of human and animal disease-causing microorganisms.

2.6 Methods to Determine Storage Stability, Shelf-life of the Microorganism

Based on the results of two separate one-year studies on *Pasteuria nishizawae* Technical and Clariva pn, both the technical and end-use product are stable when stored at 4°C for a period of 12 months.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

3.1.1 Test Studies

The PMRA conducted a detailed review of the toxicological studies submitted in support of *Pasteuria nishizawae* Technical and Clariva pn.

The studies submitted to fulfil the requirements for health hazard assessment of *Pasteuria nishizawae* Technical included acute oral toxicity/infectivity, acute pulmonary toxicity/infectivity, acute intravenous infectivity, acute dermal toxicity/irritation, dermal sensitization, and eye irritation studies.

In an acute oral toxicity study, 7-week old, Sprague-Dawley rats (12/sex) were given a single oral dose of *Pasteuria nishizawae* Technical at a dose of 5000 mg/kg bw. Over an observation period of 14 days, there were no mortalities, necropsy findings or changes in body weight. Based on this study, *Pasteuria nishizawae* Technical is of low toxicity. This oral study did not assess the infectivity potential of the MPCA.

In an acute oral infectivity and toxicity study, groups of fasted, 7-week old, CD rats (12/sex) were given a single oral dose of *Pasteuria nishizawae* Technical at a dose of 1.6×10^9 viable spores/animal and observed over a period of up to 21 days with interim sacrifices performed on Days 0, 7, 14, and 21. There were no unscheduled mortalities and no treatment related clinical signs. In this study, *Pasteuria nishizawae* Technical is of low toxicity. The potential for infectivity was not evaluated in this study, as there is no known method to recover the MPCA from animal tissues, organs or body fluids.

In an acute pulmonary infectivity and toxicity study, groups of 7-week old, CD rats (12/sex) were exposed to *Pasteuria nishizawae* Technical at a dose of 1.6×10^8 viable spores/animal by

the intratracheal route and observed over a period of up to 21 days with interim sacrifices performed on Days 0, 7, 14, and 21. There were no mortalities, treatment related clinical signs, necropsy findings or changes in body weight. Based on these results, *Pasteuria nishizawae* Technical is of low toxicity. The potential for infectivity was not evaluated in this study, as there is no known method to recover the MPCA from animal tissues, organs or body fluids.

In an acute intravenous infectivity study, groups of 7-week old, CD rats (12/sex) were injected with *Pasteuria nishizawae* Technical at a dose of 1×10^9 viable spores/animal. Animals were then observed for up to 21 days with interim sacrifices performed on Days 0, 7, 14, and 21. There were no treatment related clinical signs, necropsy findings or changes in body weight. Based on these results, *Pasteuria nishizawae* Technical is of low toxicity. The potential for infectivity was not evaluated in this study, as there is no known method to recover the MPCA from animal tissues, organs or body fluids.

In an acute dermal toxicity study, groups of young adult, New Zealand white rabbits (5/sex) were dermally exposed to *Pasteuria nishizawae* Technical at a dose of 2000 mg/kg/bw for 24 hours to an area of approximately 10% body surface area. There were no treatment related clinical signs, necropsy findings or changes in body weight. Based on this study, *Pasteuria nishizawae* Technical is of low toxicity to male and female rabbits via the dermal route.

In a dermal irritation study, young adult, New Zealand white rabbits (3females) were dermally exposed to 0.5 mL 1.6×10^9 viable spores/mL (8×10^8 viable spores/animal) of *Pasteuria nishizawae* Technical for 72 hours. Irritation was scored by the Draize method. No signs of dermal irritation (edema or erythema) were observed. Based on this study, *Pasteuria nishizawae* Technical is non- irritating to the skin of rabbits.

In a primary eye irritation study, undiluted *Pasteuria nishizawae* Technical (0.1 mL of 1.6×10^9 viable spores/mL) was instilled into the conjunctival sac of the right eye of young adult New Zealand white rabbits (3 females) and then washed after 24 hours. Irritation was scored by the method of Draize. There were no positive scores for any animal throughout the study nor were there adverse ocular effects noted. Based on this study, *Pasteuria nishizawae* Technical is non-irritating to the eye.

The studies submitted to fulfil the requirements for health hazard assessment of Clariva pn included acute oral toxicity, acute dermal toxicity, dermal and eye irritation studies.

In an oral toxicity study, young adult, Sprague-Dawley rats (5/sex) were given a single oral dose of Clariva pn at 5000 mg/kg bw. There were no treatment related clinical signs, necropsy findings or changes in body weight. Based on the results of this study, Clariva pn is of low toxicity to the rat.

In an acute dermal toxicity study, young adult, New Zealand white rabbits (5/sex) were dermally exposed to Clariva pn at a dose of 2000 mg/kg bw for 24 hours to an area of approximately 10%. There were no treatment-related clinical signs of toxicity, necropsy findings or changes in body weight. Based on this study, Clariva pn is of low toxicity in male and female rabbits via the dermal route.

In a dermal irritation study, young adult, New Zealand white rabbits (3 males) were dermally exposed to 0.5 mL of undiluted Clariva pn (2.5×10^7 viable spores/mL) for 4 hours. Animals then were observed for 72 hours. Irritation was scored by the method of Draize. There were no dermal effects were observed throughout the study. Based on this study, Clariva pn is non-irritating to the skin.

In a primary eye irritation study, undiluted Clariva pn (0.1 mL of 2.5×10^7 viable spores/mL) was instilled into the conjunctival sac of the right eye of young adult New Zealand white rabbits (3 males) and then washed after 24 hours. Irritation was scored by the method of Draize. There were no positive scores for any animal throughout the study nor were there adverse ocular effects noted. Based on this study, Clariva pn is non-irritating to the eye.

Results of the toxicity and infectivity of *Pasteuria nishizawae* Technical and acute toxicity of its associated end-use product, Clariva pn, are summarised in Appendix I, Table 1.

3.1.2 Additional Information

Acceptable waiver rationales were submitted to waive hypersensitivity testing for *Pasteuria nishizawae* Technical and Clariva pn. The rationales were based on a lack of hypersensitivity effects observed in workers and researchers.

3.1.3 Incident Reports Related to Human and Animal Health

Since April 26, 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website. Incidents were reviewed for the active ingredient *P. nishizawae* Pn1. As of February 24, 2014, no incident reports involving *P. nishizawae* Pn1 have been reported to the PMRA.

3.1.4 Hazard Analysis

The database submitted in support of registering *Pasteuria nishizawae* Technical and Clariva pn was reviewed from the viewpoint of human health and safety and was determined to be sufficiently complete to permit a decision on registration.

Pasteuria nishizawae Technical was of low toxicity to rats via the oral, pulmonary and intravenous routes; and to rabbits via the dermal route. Although the infectivity studies were conducted according to guidelines, infectivity could not be properly evaluated since clearance could not be confirmed. However, additional infectivity testing will not be required given the host-specific mode of action of the MPCA and that no clinical signs of infection were observed via the oral, pulmonary and intravenous routes. In addition, *Pasteuria nishizawae* Technical showed no dermal or eye irritation in the rabbit.

The end-use product was of low toxicity to rats via the oral route, and of low toxicity to rabbits via the dermal route. No dermal or ocular irritation was observed in rabbits treated with Clariva pn.

Although there is reportedly no hypersensitivity effects noted in workers or researchers handling *Pasteuria nishizawae* Technical or Clariva pn, the signal words "POTENTIAL SENSITIZER" will appear on the respective labels, as all microorganisms are recognized as being able to produce substances that can elicit allergic reactions after repeated exposure to high concentrations.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of Clariva pn, and no indications of infectivity, toxicity or pathogenicity of *P. nishizawae* Pn1 in the test animals treated in the Tier I acute oral, pulmonary, intravenous, toxicity/infectivity tests.

Within the available scientific literature, there are no reports that suggest *P. nishizawae* Pn1 has the potential to cause adverse effects on the endocrine system of animals. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *P. nishizawae* Pn1.

3.2 Occupational, Residential and Bystander Risk Assessment

3.2.1 Occupational Exposure and Risk

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with primary exposure routes being dermal and inhalation. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Pasteuria nishizawae* Pn1 has not been identified as a dermal wound pathogen and does not contain any known toxic secondary metabolites. There is no indication that it could penetrate intact skin of healthy individuals. Furthermore, toxicity testing with *Pasteuria nishizawae* Technical and Clariva pn showed no significant signs of toxicity via the oral, dermal, intravenous or pulmonary routes of exposure. The submitted eye and dermal irritation studies with the *Pasteuria nishizawae* Technical and Clariva pn demonstrated minimal eye and skin irritation.

The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Therefore, anyone handling or applying Clariva pn must wear a long-sleeved shirt, long pants, shoes plus socks, waterproof gloves, and a NIOSH approved mist filtering mask or respirator with any N-95, P-95 or R-95 filter.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of Clariva pn, and no significant occupational risks are anticipated for this product.

3.2.2 Residential and Bystander Exposure and Risk

Adults, youths and toddlers are unlikely to be exposed to *P. nishizawae* Pn1 as Clariva pn is to be used as a seed treatment. In the event of exposure, the PMRA does not expect that residential and bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity

profile for *P. nishizawae* Pn1 and Clariva pn. It is also assumed that precautionary label statements will be followed by commercial applicators in the use of Clariva pn. As well, *P. nishizawae* Pn1 is a species that is ubiquitous in the environment and the use of Clariva pn is not expected to cause sustained increases in exposure to bystanders beyond natural levels. Consequently, the health risk to infants and children is expected to be negligible.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

The proposed use pattern is not expected to result in dietary exposure, and thus, risk is expected to be of no concern for the general population, including infants and children, or animals because *P. nishizawae* Pn1 demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity/infectivity study. Higher tier subchronic and chronic dietary exposure studies were not required because of the low toxicity of the MPCA and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral, intravenous and pulmonary toxicity/infectivity studies. Therefore, there are no concerns for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.3.2 Drinking Water

No risks are expected from exposure to this microorganism via drinking water because the use pattern exposure will be minimal and there were no harmful effects observed in Tier I acute oral toxicity testing and infectivity testing. The Clariva pn label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Furthermore, municipal treatment of drinking water is expected to remove the transfer of residues to drinking water. Therefore, potential exposure to *P. nishizawae* Pn1 in surface and drinking water is negligible.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARfDs) and acceptable daily intakes (ADIs) are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e. no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that *P. nishizawae* Pn1 is of low toxicity, is not infective to mammals, and that infants and children are likely to be no more sensitive to the MPCA than the general population. Thus, there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCA and other

registered micro-organisms that have a common mechanism of toxicity, does not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *P. nishizawae* Pn1 to human health.

3.3.4 Aggregate Exposure and Risk

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA's files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *P. nishizawae* Pn1 to the general Canadian population, including infants and children, when Clariva pn is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Furthermore, few adverse effects from exposure to other isolates of *P. nishizawae* encountered in the environment have been reported. Even if there is an increase in exposure to this active ingredient from the use of Clariva pn, there should not be any increase in potential human health risk.

3.3.5 Maximum Residue Limits

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally specified as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada specifies science-based MRLs to ensure the food Canadians eat is safe.

Residues of *P. nishizawae* Pn1 on treated food crops, at the time of harvest, are not anticipated following seed treatment. The PMRA has applied a hazard-based approach for determining whether an MRL is required for this microorganism. No adverse effects from dietary exposure have been attributed to natural populations of *P. nishizawae*, and no adverse effects were observed in the acute oral toxicity conducted with *P. nishizawae* Pn1. In addition, the likelihood of residues contaminating drinking water supplies is negligible and not of concern. Therefore, the PMRA has determined that the specification of an MRL under the *Pest Control Products Act* is not required for *P. nishizawae* Pn1.

3.4 Cumulative Effects

The PMRA has considered available information on the cumulative effects of residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Besides naturally occurring strains of *P. nishizawae* in the environment, the PMRA is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity with *P. nishizawae* Pn1. No cumulative effects are anticipated if the residues of *P. nishizawae* Pn1 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Pasteuria spp. are ubiquitous spore producing bacteria, found in more than 51 countries on five continents and on various islands in the Atlantic, Pacific and Indian oceans. They are known for their ability to disrupt reproduction in their nematode hosts and have been shown to reduce nematode damage on affected crops. The spores are stable in a wide range of environmental conditions and, with the exception of *P. ramosa* that parasitizes daphnids, attach to and parasitize host plant-parasitic nematodes. The population dynamics of members of the genus *Pasteuria* is tightly linked to its nematode host in a typical parasite/prey relationship. Crops susceptible to nematodes foster higher population densities of *Pasteuria* spp., which ultimately serve to lower the nematode population.

Pasteuria nishizawae Pn1 was isolated from soil samples collected in the southern United States. It produces spores that attach to and infect its host soybean cyst nematode, *Heterodera glycines*. Aside from eggs, the endospores attach to all nematode stages, from 2nd stage juveniles (J2) through adults, but will not germinate until the nematodes burrow into the soybean root. This infection cycle only occurs in female nematodes. Spores of *P. nishizawae* Pn1 will only parasitize cyst nematodes in the genus *Heterodera*.

The levels of *P. nishizawae* Pn1 spores in the terrestrial and aquatic environment are not expected to significantly increase as a result of the use of Clariva pn as a soybean seed treatment.

4.2 Effects on Non-Target Species

PMRA has a four-tiered approach to environmental testing of microbial pesticides. Tier I studies consist of acute studies on up to seven broad taxonomic groups of non-target organisms exposed to a maximum hazard or Maximum Challenge Concentration (MCC) of the MPCA. The MCC is generally derived from the amount of the MPCA, or its toxin, expected to be available following application at the maximum recommended label rate multiplied by a safety factor. Tier II studies consist of environmental fate (persistence and dispersal) studies as well as additional acute toxicity testing of MPCAs. Tier III studies consist of chronic toxicity studies (i.e. life cycle studies) as well as definitive toxicity testing (for example, LC_{50}, LD_{50}). Tier IV studies consist of experimental field studies on toxicity and fate, and are required to determine whether adverse effects are realized under actual use conditions.

The type of environmental risk assessment conducted on MPCAs varies depending on the tier level that was triggered during testing. For many MPCAs, Tier I studies are sufficient to conduct environmental risk assessments. Tier I studies are designed to represent "worst-case" scenarios where the exposure conditions greatly exceed the expected environmental concentrations. The absence of adverse effects in Tier I studies are interpreted as minimal risk to the group of non-target organisms. However, higher tiered studies will be triggered if significant adverse effects on non-target organisms are identified in Tier I studies. These studies provide additional information that allows PMRA to refine the environmental risk assessments. In the absence of adequate environmental fate and/or field studies, a screening level risk assessment can be

performed to determine if the MPCA is likely to pose a risk to a group of non-target organisms. The screening level risk assessment uses simple methods, conservative exposure scenarios (for example, direct application at a maximum application rate) and sensitive toxicity endpoints. A risk quotient (RQ) is calculated by dividing the exposure estimate by an appropriate toxicity value (RQ = exposure/toxicity), and the risk quotient is then compared to the level of concern (LOC).

If the screening level risk quotient is below the level of concern, the risk is considered negligible and no further risk characterization is necessary. If the screening level risk quotient is equal to or greater than the level of concern, then a refined risk assessment is performed to further characterize the risk. A refined assessment takes into consideration more realistic exposure scenarios (environmental fate and/or field testing results). Refinements to the risk assessment may continue until the risk is adequately characterized or no further refinements are possible.

4.2.1 Effects on Terrestrial Organisms

In lieu of guideline studies to address the hazards of the technical grade active ingredient to terrestrial non-target organisms, acceptable scientific rationales were submitted to waive Tier I testing requirements. The rationales were based on the host specificity of the MPCA and lack of adverse effects in the published scientific literature. In addition, *Pasteuria nishizawae* Pn1 is ubiquitous in soil, and levels in the environment are not expected to significantly increase above background levels when Clariva pn is applied as a seed treatment in accordance with proposed label use directions. Furthermore, there were no treatment related effects in acceptable mammalian toxicological studies conducted with the technical grade active ingredient and end-use product.

An independent search of the scientific literature using keywords through PubMed search engine yielded no reports of adverse effects to birds, plants, wild mammals, arthropods and non-arthropod invertebrates other than its intended host, *H. glycines*.

Based on an absence of information in the public domain on the adverse effects of *P. nishizawae* Pn1 to non-target terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, terrestrial arthropods and non-arthropod invertebrates, and terrestrial plants from the proposed use of Clariva pn as a soybean seed treatment.

4.2.2 Effects on Aquatic Organisms

No studies were submitted to address the hazards of the technical grade active ingredient to aquatic non-target organisms. In scientific rationales submitted to waive data requirements for testing on aquatic non-target organisms, it was stated that members of the genus *Pasteuria* are host specific to plant parasitic nematodes including the MPCA which is highly specific to the soybean cyst nematode. However one species, *P. ramosa*, is known to parasitize daphnids but does not attach to or infect nematodes. No other reports of adverse effects were found on non-target aquatic organisms, and based on the terrestrial use pattern no significant exposure to aquatic systems is expected.

An independent search of scientific literature using keywords through PubMed search engine yielded no reports of adverse effects to fish, aquatic arthropods and non-arthropod invertebrates, and aquatic plants other than its intended host, *H. glycines*.

Based on an absence of information in the public domain on the adverse effects of *P. nishizawae* Pn1 to non-target aquatic organisms, there is reasonable certainty that no harm will be caused to fish, aquatic arthropods and non-arthropod invertebrates, and aquatic plants from the proposed use of Clariva pn. As a general precaution, the label will direct handlers to not contaminate surface water by disposal of equipment wash waters.

4.3 Incident Reports related to the Environment

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website. Only incidents in which the pesticide is determined to be linked to the effects (Canadian causality of highly probable, probable and possible; American causality of highly probable, probable and possible) are considered in the reviews.

As of 10 February 2014, there were no environmental incidents reported in the PMRA Incident reporting database for products containing *P. nishizawae* Pn1 for use as pesticides.

5.0 Value

5.1 Effectiveness Against Pests

Efficacy data from two trials, conducted in 2010 in Wisconsin, were provided where Clariva pn was applied at a rate of 10 million spores per seed. The treatment showed a statistically significant decrease in the number of cysts per root, with a reduction of 53-63%. Yield data from four trials, conducted from 2011-2013, in Iowa, Illinois, Nebraska and Ontario, showed statistically significant increases (7-15%) at 10 million spores per seed, thus confirming the observations from the efficacy data.

5.2 Non-Safety Adverse Effects

No phytotoxicity was reported in host plants in any of the trials for which efficacy data were provided.

5.3 Consideration of Benefits

5.3.1 Social and Economic Impact

Clariva pn is registered in the United States. Registration of this product in Canada, will ensure that soybean producers in Canada have equal access to this soybean cyst nematode management tool.

Clariva pn may have an indirect effect on reducing the impact of other soil borne pests, by reducing the level of feeding on soybean roots, thereby reducing the entry points for pathogens like *Fusarium virguliforme* to enter. This pathogen causes Sudden Death Syndrome, which can be a devastating disease as few management strategies are currently available to growers.

Soybean cyst nematode was first identified in Ontario in 1988, and has been identified in most counties west of Toronto and more recently in parts of Eastern Ontario (Ontario Ministry of Agriculture, Food and Rural Affairs website). In 2013, SCN was identified in Quebec for the first time. Once SCN is in a field, eradication is impossible. With an integrated pest management strategy, which includes a biological nematicide such as Clariva pn, the use of SCN resistant varieties, and rotation to non-host crops, growers can keep SCN populations under control.

5.3.2 Survey of Alternatives

Other than fumigants that are registered for the control of nematodes for soybean in storage, the only alternative available for the management of soybean cyst nematode is Votivo 240 FS (Registration number 30279), containing *Bacillus firmus* strain I-1582, another biological product. This product is also a seed treatment that provides suppression of SCN.

5.3.3 Compatibility with Current Management Practices Including Integrated Pest Management

The active ingredient, *Pasteuria nishizawae* Pn1, is a naturally occurring soil microorganism. Clariva pn can be part of an integrated pest management strategy, which includes the use of SCN resistant varieties and rotation to non-host crops.

5.3.4 Information on the Occurrence or Possible Occurrence of the Development of Resistance

The risk for resistance development to Clariva pn is low given that this is a biological product. Furthermore, the use of Clariva pn could help delay the development of susceptibility of resistant soybean varieties to SCN, as soybean cyst nematodes have the ability to adapt to the source of resistance found in resistant soybean varieties (for example, varieties with the P188788 trait).

5.4 Supported Uses

Based on the value information provided a claim of suppression of soybean cyst nematode (*Heterodera glycines*), at an application rate of 10 million spores per seed, can be supported.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The Toxic Substances Management Policy (TSMP) is a federal government policy developed to provide direction on the management of substances of concern that are released into the environment. The TSMP calls for the virtual elimination of Track 1 substances [those that meet all four criteria outlined in the policy, i.e. persistent (in air, soil, water and/or sediment),

bio-accumulative, primarily a result of human activity and toxic as defined by the *Canadian Environmental Protection Act].*

Pasteuria nishizawae Technical and Clariva pn were assessed in accordance with the PMRA Regulatory Directive DIR99-03.[1]

- *Pasteuria nishizawae* Technical does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.
- There are also no formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track-1 criteria.

6.2 Formulants and Contaminants of Health or Environmental Concern

During the review process, contaminants in the technical and formulants and contaminants in the end-use product are compared against the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* maintained in the *Canada Gazette*.[2] The list is used as described in the PMRA Notice of Intent NOI2005-01[3] and is based on existing policies and regulations including: DIR99-03, and DIR2006-02[4] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

- *Pasteuria nishizawae* Technical and Clariva pn do not contain any other formulants or contaminants of environmental concern identified in the Canada Gazette, Part II, Volume 139, Number 24, pages 2641-2643: List of Pest Control Product Formulants of Health or Environmental Concern

The use of formulants in registered pest control products is assessed on an ongoing basis through PMRA formulant initiatives and DIR2006–02.

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1 Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy.*

2 *Canada Gazette*, Part II, Volume 139, Number 24, SI/2005-11-30) pages 2641-2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* and in the order amending this list in the *Canada Gazette*, Part II, Volume 142, Number 13, SI/2008-67 (2008-06-25) pages 1611-1613: *Part 1 Formulants of Health or Environmental Concern, Part 2 Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions and Part 3 Contaminants of Health or Environmental Concern.*

3 Notice of Intent NOI2005-01, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern under the New Pest Control Products Act.*

4 Regulatory Directive DIR2006-02, *Formulants Policy and Implementation Guidance Document.*

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for *Pantoea reticulata* Technical and Clariva pn were judged to be adequate to assess their potential human health and environmental risks. The technical grade active ingredient was characterized and the specifications were supported by the analyses of a sufficient number of batches. Storage stability data were sufficient to support a shelf life of up to 12 months for *Pantoea reticulata* Technical and Clariva pn when stored at a temperature of 4°C.

7.2 Human Health and Safety

The acute toxicity and infectivity studies and other relevant information submitted in support of *P. reticulata* Pn1 and Clariva pn were determined to be sufficiently complete to permit a decision on registration. Submitted information suggests *Pantoea reticulata* Technical to be of low toxicity and not pathogenic by the oral, pulmonary, intravenous, and dermal routes. Although the potential for infectivity could not be properly evaluated, no additional infectivity testing are required given the host specificity of the MEPCA and the absence of any signs of infections in animals via the oral, pulmonary and intravenous injection routes of exposure. Also, Clariva pn was low in toxicity/irritation via the oral, ocular and dermal routes of exposure in animals. Both, *Pantoea reticulata* Technical and Clariva pn are considered potential sensitizers such that "POTENTIAL SENSITIZER" is required on the principal display panel of both products.

When handled according to prescribed label instructions, the potential for dermal, eye and inhalation exposure for mixer/loaders, applicators, and handlers exists, with the primary source of exposure to workers being dermal and to a lesser extent inhalation.

In individuals repeatedly exposed to large quantities of Clariva pn, respiratory and dermal sensitivity could possibly develop since all microorganisms, including *P. reticulata* Pn1, contain substances that are potential sensitizers. Therefore, anyone handling or applying Clariva pn must wear waterproof gloves, long-sleeved shirts, long pants, shoes plus socks, and a NIOSH approved mist filtering mask or respirator with any N-95, P-95 or R-95 filter.

The health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is expected to be minimal since Clariva pn will only be applied as a seed treatment to soybeans. The product is not to be applied to residential or recreational areas. The specification of an MRL under the *Pest Control Products Act* is not required for *P. reticulata* Pn1.

7.3 Environmental Risk

The scientific rationales and published scientific literature submitted in support of *Pantoea reticulata* Technical and Clariva pn were determined to be sufficiently complete. The seed treatment use of Clariva pn is not expected to pose a risk to non-target organisms when the directions for use on the label are followed.

As a general precaution, the label will also prohibit the direct application of Clariva pn to aquatic habitats (such as lakes, rivers, sloughs, ponds, prairie potholes, creeks, marshes, streams, reservoirs, and wetlands), estuaries or marine habitats, and require handlers to not contaminate surface water by disposal of equipment wash waters.

No other environmental fate studies or non-target organism studies are required to consider a decision on the registration of Clariva pn for use as a seed treatment on soybeans.

7.6 Value

The data submitted to register Clariva pn are adequate to demonstrate value for use on soybean in suppressing SCN (*Heterodera glycines*). Clariva pn, as part of an Integrated Pest Management system, will contribute to the management of SCN for which only one other alternative product exists.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Pasteuria nishizawae* Technical and Clariva pn, containing the technical grade active ingredient *Pasteuria nishizawae* Pn1, to suppress soybean cyst nematode in soybean.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

µl	microlitre(s)
°C	degree(s) Celsius
ADI	acceptable daily intake
ARfD	acute reference dose
bw	body weight
CD	Charles Darwin
cv%	Coefficient(s)
DACO	data code
DIR	Regulatory Directive
EP	end-use product
FS	flowable suspension
g	gram(s)
IPM	integrated pest management
kg	kilogram(s)
KTG	killed treatment group
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOC	level of concern
MCC	Maximum Challenge Concentration
mg	milligram(s)
ml	millilitre(s)
MIS	maximum irritation score
MPCA	microbial pest control agent
MRL	maximum residue limit
NIOSH	National Institute for Occupational Safety and Health
NOI	Notice of Intent
PMRA	Pest Management Regulatory Agency
PubMed	global public domain database on life sciences and biomedical topics which is maintained by the United States National Library of Medicine
RQ	risk quotient
rRNA	ribosomal ribonucleic acid
SCN	soybean cyst nematode
Spp	species
TG	treatment group
TGAI	technical grade of the active ingredient
TSMP	Toxic Substances Management Policy
w/w	weight/weight

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *Pasteuria nishizawae* Technical and its associated end-use product, Clariva pn.

Study Type	Species, Strain, and Doses	Results	Comments	Reference(s)
Acute Toxicity/Infectivity of *Pasteuria nishizawae* Technical				
Acute Oral Toxicity (14-Day study)	Rat - Sprague-Dawley 12/sex, single oral dose, 5000 mg/kg bw	LD_{50} > 5000 mg/kg bw	There were no mortalities and no treatment related clinical signs throughout the study period. **LOW TOXICITY** **ACCEPTABLE**	2113162
Acute Oral Infectivity and Toxicity (21-Day study)	Rat - CD® Treatment group (TG): 12/sex, 1.0 mL at 1.6×10^7 viable spores per animal. Interim sacrifices on Days 0, 7, 14 and 21 Killed treatment group (KTG) 3/sex, 1.0 mL/animal), 3/sex, untreated, naive control 3/sex, untreated – shelf control Body weights measured on Days 0, 7, 14 and 21.	LD_{50} > 1.6×10^7 viable spores/animal	There were no mortalities. There were no treatment related clinical signs, necropsy findings or changes in body weight. Clearance not assessed **LOW TOXICITY,** **ACCEPTABLE FOR TOXICITY ONLY**	2113160
Acute Pulmonary Infectivity and Toxicity (21-Day study)	Rat - CD® Treatment group (TG): 12/sex, 1.6×10^8 spores per animal in 0.1 mL dose. Interim sacrifices on Days 0, 7, 14 and 21 Killed treatment group (KTG) 3/sex, 0.1 mL/animal), 3/sex, untreated, naive control Body weight measured	LD_{50} > 1.6×10^8 viable spores/animal	There were no mortalities. There were no treatment related clinical signs, necropsy findings or changes in body weight. Clearance not assessed **LOW TOXICITY,** **ACCEPTABLE FOR TOXICITY ONLY**	2113166

Study Type	Species, Strain, and Doses	Results	Comments	Reference(s)
	on Days 0, 7, 14, and 21.			
Acute Intraperitoneal Infectivity (21-Day study)	Rat - CD 12/sex, intraperitoneal injection, 1×10^9 spores/animal in 0.7 mL dose; Interim sacrifices on Days 0, 7, 14 and 21. Killed treatment group (KTG) 3/sex, 0.7 mL/animal), 3/sex, untreated, naïve control Body weights measured on Days 0, 7, 14 and 21	$LD_{50} > 1 \times 10^9$ viable spores/animal	No mortalities or effect on bodyweight gain, and no clinical signs of treatment-related toxicity, infectivity or pathogenicity. No necropsy findings. Clearance not assessed. **LOW TOXICITY,** **ACCEPTABLE FOR TOXICITY ONLY**	2113169
Acute Dermal Toxicity (14-Day study)	Rabbit- New Zealand 5/sex, 24 hour dermal exposure, 2000 mg/kg bw Body weight measured on Days 0, 7 and 14	$LD_{50} > 2000$ mg/kg bw	There were no mortalities, treatment related clinical signs, necropsy findings or changes in body weight **LOW TOXICITY** **ACCEPTABLE**	2113172
Acute Irritation/Sensitization of *Pasteuria nishizawae* Technical				
Dermal Irritation	Rabbit-New Zealand white 3 females, 4-hour dermal exposure, 0.5 mL at 1.6×10^9 spores/mL Observed for 3 days	MIS=0	No dermal irritation occurred in any rabbit during the study. **NON IRRITATING** **ACCEPTABLE**	2259569
Eye Irritation	Rabbit-New Zealand white 3 females 0.1 mL at 1.6×10^9 spores/mL, 24 hour exposure	MIS= 0	No ocular irritation occurred in any of the rabbits. **NON-IRRITATING** **ACCEPTABLE**	2259570
Acute Irritation of Clariva pn				
Dermal Irritation	Rabbit-New Zealand white 3males 0.5 mL at 2.5×10^7 spores/mL 72 hour exposure	MIS=0	No edema or dermal effects were observed throughout the study. **NON-IRRITATING** **ACCEPTABLE**	2259572

Study Type	Species, Strain, and Doses	Results	Comments	Reference(s)
Eye Irritation	Rabbit-New Zealand white 3 males, 0.1 mL at 2.5×10^{7} spores/mL instilled in right eye for 24 hours. The treated eye was washed 30 seconds after instillation	MIS= 0	No ocular effects were observed in any of the rabbits on the study. **NON-IRRITATING** **ACCEPTABLE**	2259703
Acute Toxicity of Clariva pn				
Acute Oral Toxicity (14-Day study)	Rat-Sprague-Dawley 5/sex, single oral dose of 5000 mg/kg bw (2.5×10^{7} spores/mL); Body weight measured on Days 0, 7 and 14.	LD_{50}> 5000 mg/kg bw	No treatment related clinical signs, necropsy findings or changes in body weight. **LOW TOXICITY,** **ACCEPTABLE**	2259698
Acute Dermal Toxicity (14-Day study)	Rabbit- New Zealand white 5/sex, 24-hour dermal exposure (area of 10%), 2000 mg/kg bw Body weight measured on Days 0, 7 and 14	LD_{50}> 2000 mg/kg bw	There were no mortalities. There were no treatment-related clinical signs of toxicity, necropsy findings or changes in body weight. **LOW TOXICITY** **ACCEPTABLE**	2259701

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Organisms				
Vertebrates				
Birds	A request to waive the requirement for test data was submitted based on the host specificity of the MPCA and that no reports of adverse effects were found in published scientific literature. No further data are required to assess the risk of harm to birds.			2113182
Wild Mammals	From the data submitted under the Part M4 Human Health and Safety Testing, it was determined that the TGAI and EP were not toxic or pathogenic to mammals via the oral, pulmonary, dermal, intravenous or intraperitoneal routes. No further data are required to assess the risk of harm to non-target wild mammals.			2384666

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Invertebrates				
Arthropods				
Terrestrial Arthropods	A request to waive the requirement for test data was submitted based on the host specificity of the MPCA and that no reports of adverse effects were found in published scientific literature. No further data are required to assess the risk of harm to terrestrial arthropods.			2113182
Non-arthropods				
Terrestrial Non-Arthropod Invertebrates	A request to waive the requirement for test data was submitted based on the host specificity of the MPCA and that no reports of adverse effects were found in published scientific literature. Members of the genus *Pasteuria* are not known to infect beneficial nematodes. No further data are required to assess the risk of harm to non-target terrestrial non-arthropod invertebrates.			2113182
Plants				
Plants	A request to waive the requirement for test data was submitted. There are no reports of negative effects on plants in published scientific literature. No further data are required to address the hazard of *P. nishazawae* Pn1 to terrestrial plants.			2384666
Microorganisms				
Micro-organisms	A request to waive the requirement for test data was not submitted. No further data are required to assess the risk of harm to microorganisms.			
Aquatic Organisms				
Vertebrates				
Fish	A request to waive the requirement for test data was submitted based on the host specificity of the MPCA and that no reports of adverse effects were found in published scientific literature. No further data are required to assess the risk of harm to fish.			2113182
Invertebrates				
Aquatic Arthropods	A request to waive the requirement for test data was submitted based on the host specificity of the MPCA, although one species of *Pasteuria*, *P. ramosa*, is known to parasitize daphnids. There are no other reports of adverse effects found in published scientific literature. No further data are required to assess the risk of harm to aquatic arthropods.			2113182
Aquatic Non-Arthropod Invertebrates	A request to waive the requirement for test data was submitted based on the host specificity of the MPCA and that no reports of adverse effects were found in published scientific literature. No further data are required to assess the risk of harm to aquatic non-arthropod invertebrates.			2113182
Plants				
Aquatic Plants	A request to waive the requirement for test data was submitted based on the host specificity of the MPCA and that no reports of adverse effects were found in published scientific literature. No further data are required to assess the risk of harm to aquatic plants.			2384666

References

A. List of Studies/Information Submitted by Registrant

1.0 Chemistry

PMRA Document Number	Reference
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2.0 Human and Animal Health

PMRA Document Number	Reference
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2113176	2010, Volume 13: Hypersensitive Incidents *Pasteuria nishizawae* Pn1. DACO: M4.6
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2015070	2010, Volume 11 of EUP: Acute Dermal Toxicity Study of *Fraxinus mikinorae* Pst1 (end Product) in Rabbits. DACO: M4.4
2015076	2010, Volume 13 of EUP: Acute Dermal Irritation Study of *Fraxinus mikinorae* Pst1 (end Product) in Rabbits. DACO: M4.5.2
2015077	2010, Volume 5 of EUP: Hypersensitive Incidents *Fraxinus mikinorae* Pst1 (Liquid Formulation, End Product). DACO: M4.6
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2384692	2008, Copies of Publications. DACO: M2.1.6, M4.9, M9.9
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2384709	2010, Clariva pn (EUP) Alternate Name: *Fraxinus mikinorae* - Pst1 (Liquid Formulation, EP) Human Health and Safety Toxicology Testing - Summary. DACO: M4.1, M4.2.1, M4.9.1
2384668	2012, Biopesticides Registration Action Document *Fraxinus mikinorae* Pst1. DACO: M10.2.9

5.0 Environment

PMRA Document Number	Reference
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2384666	2014, Request for Waivers from the Requirement to Conduct Studies - M9.3 Wild Mammals, M9.5.1 Terrestrial Plants, M9.6.2 Aquatic Plants (Tier 1). DACO: M9.3, M9.4.1, M9.4.2

6.0 Value

PMRA Document Number	Reference
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2384720	2012, Evaluating Experimental Nematicides on Soybean: Mixtures of Greenhouse and Microplot. DACO: M10.2.2

2384721 2011, Broad Geography Testing of Avicta Complete Soybean in the Western Corn Belt (Sting Nematode), DACO: M10.2.2

2384723 2011, Avicta Complete Soybean - Broad Geography Testing (BAT) Using Soybean Differentials and Yield Effects, DACO: M10.2.2

2384724 2011, Broad Geography Testing of Avicta Complete Soybean in the Western Corn Belt (Sting Nematode), DACO: M10.2.2

2384725 Evaluate Clariva Seed treatment (A19824C) for Control of Soybean Cyst Nematode in Soybean, DACO: M10.2.2

B. Additional Information Considered

i) Published Information

1.0 Chemistry

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